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Review Article

CELLULAR APOPTOSIS AND ORGAN INJURY IN SEPSIS: A REVIEW

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APOPTOSIS: PHYSIOLOGICAL CELL DEATH

Apoptosis, as a biological phenomenon, is readily identifiable by several characteristic features. It characteristically affects scattered single cells, not groups of contiguous cells as in necrosis, with the dying cell undergoing a relatively ordered form of cell death. This physiological cell death is characterized by cell shrinkage, cellular crenation, cytoplasmic and chromatin condensation, and internucleosomal DNA fragmentation (1). Changes in membrane glycosylation and lipid profiles, and alteration in expression of surface receptors have been observed. The apoptotic cells are rapidly phagocytosed and degraded by neighbouring cells or resident macrophages without an inflammatory response. This mechanism prevents the release of the phlogistic contents of cells and avoids the possibility of neighbouring host cell injury. The process differs significantly from cell death by necrosis or lysis where cells release their contents into the surrounding tissues and perpetuate the local inflammatory response. A glossary of terms pertinent to this review is included as an appendix.

APOPTOSIS: MORPHOLOGICAL EVENTS

During apoptosis, the dying cell undergoes a series of profound structural changes. The earliest event observed by electron microscopy is condensation of chromatin to form sharply circumscribed, uniformly dense, cresentic masses that abut the nuclear envelope (2). Nucleolar changes include the dispersal of peripheral nucleolar chromatin to form aggregates in the centre of the nucleus. Simultaneously with the nuclear changes, apoptotic cells detach from neighbouring cells, and specialized surface structures such as microvilli appear. Cell volume decreases, cell density increases, cytoplasmic organelles compact, and convolution of the cell and nuclear outline becomes evident (1) (Fig. 1). Cytoplasmic changes include cytoskeletal filament aggregation, clumping of ribosomal particles, and rearrangement of rough endoplasmic reticulum to form a series of concentric whorls (3). Cytoplasmic and nuclear condensation is followed by the production of numerous membrane protuberances at the plasma membrane that subsequently separate with sealing of the plasmalemma to form membrane-bound apoptotic bodies of varying sizes with condensed cytoplasm and crowded, intact cytoplasmic organelles. The production of apoptotic bodies is a late occurrence in the apoptotic process and is observed extensively *in vitro*, but less commonly *in vivo*. This observation emphasises the rapidity of the apoptotic process whereby apoptotic cells are rapidly phagocytosed *in vivo* prior to apoptotic body formation (4). Phagocytosis is mediated by adjacent epithelial cells, mononuclear phagocytes, or tumor cells. Once phagocytosed, apoptotic bodies are degraded by lysosomal enzymes derived from the ingesting cell. Rapid phagocytosis of apoptotic cells *in vivo* before their secondary degeneration helps explain the absence of inflammation associated with apoptosis. Apoptotic bodies that escape phagocytosis lose their integrity after an hour or so, resulting in swelling, loss of density, membrane rupture, and organelle disruption and dispersal referred to as secondary necrosis (Fig. 1).

APOPTOSIS: BIOCHEMICAL EVENTS

Cytoskeletal and membrane alterations

Cell shrinkage and apoptotic body formation require significant changes in both the cytoskeleton and plasma membrane lipid bilayer. Cytoskeletal changes include tissue transglutaminase activation, microtubule disruption, α-fodrin (nonerythroid spectrin) and actin cleavage, and a requirement for actin polymerization. These changes facilitate membrane budding and play a role in the maintenance of plasma membrane integrity in apoptotic cells (5). Membrane changes include redistribution of phosphatidylserine from its normal location on the inner leaf of the plasma membrane lipid bilayer to the outer leaf, exposure of surface sugar residues from loss of membrane sialic acid, and loss of expression of surface markers such as FcγRIII (CD16), complement regulatory molecules (CD45 and CD59), and adhesion molecules (CD11/CD18). These membrane changes are believed to play a role in the recognition and eventual phagocytosis of apoptotic cells (6).

Cell shrinkage

Condensation of the cytoplasmic space resulting in cell shrinkage appears to be a universal characteristic of apoptosis (7). This change is thought to be consequent to net movement of water out of the cell due to vesicles budding from the endoplasmic reticulum and Golgi apparatus fusing with the plasma membrane with release of their contents into the extracellular space. A role for active ion efflux has been implicated in cell shrinkage with active efflux of Na^+ and K^+ ions through the Na^+,K^+ ATPase pump and Ca^{2+}-dependent channel (8, 9).

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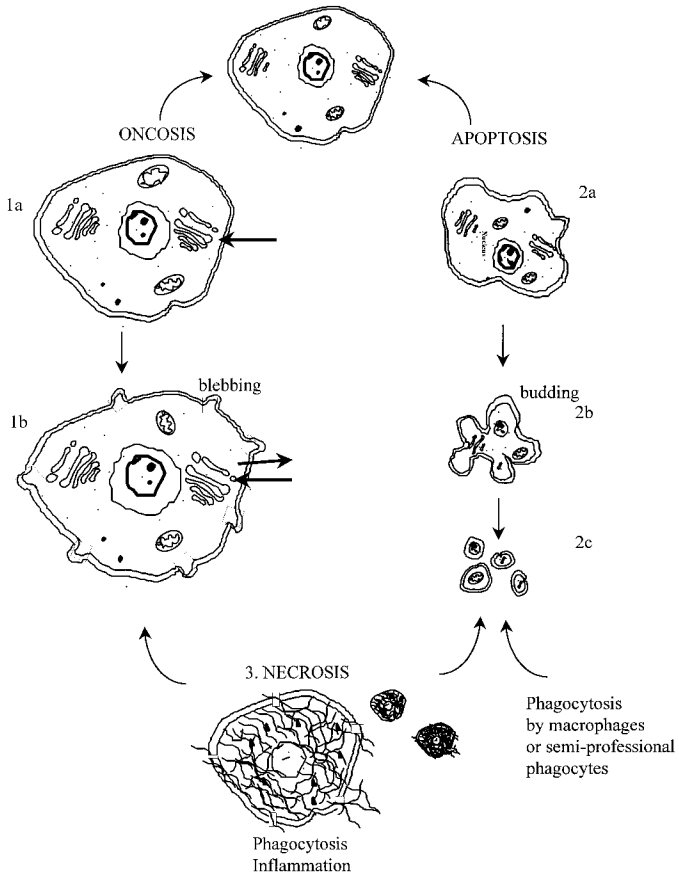

Fig. 1. **Morphological aspects of cell death by oncosis and apoptosis.** Necrosis can occur after both forms of cell death. A normal cell is shown at the top. 1a, Swelling. 1b, Blebbing, vacuolization, and increased permeability. 2a, Shrinkage and pyknosis. 2b, Budding and karyohexis. 2c, Apoptotic bodies. 3, Necrotic changes (shrinkage, coagulation, and karyolysis) occurring after rupture of a cell surface bleb in oncosis, or secondarily due to failure of apoptotic bodies to be phagocytosed in apoptosis. Adapted from Majno and Joris, 1995.

Apoptotic DNA degradation

Apoptotic cells display dramatic changes in the nucleus, including chromatin condensation and margination. A further nuclear-associated event during apoptosis is the degradation of DNA into 180- to 200-bp oligonucleosomal fragments. These fragments form a ladder-type pattern when subjected to agarose gel electrophoresis, a feature that is now one of the biochemical hallmarks of apoptosis (10). However, it should be noted that apoptotic internucleosomal DNA fragmentation is not universal, although higher molecular weight fragments (50–300 Kbp) have been reported in certain cell types immediately proceeding or in the absence of oligonucleosomal fragmentation (11). A number of putative endonucleases have been proposed, including, DNase I, DNase II, NUC-18, as well as other novel endonucleases including the caspase-activated deoxyribonuclease (12). The fact that mRNA of these endonucleases is expressed in only a limited number of human tissues suggests that other enzymes may participate in the degradation of DNA during apoptosis.

Regulators of apoptosis

It is well established that cell proliferation and differentiation are highly regulated processes; however, it is now emerging that regulation of cell death is just as complex and equally important in the maintenance of tissue homeostasis (13). Apoptotic cell death is regulated by genetic factors, and the intrinsic death program can be modulated by exogenous "survival" factors.

Despite Kerr's seminal work (14) revealing that most physiological forms of cell death share a common set of morphological features, and the assumption that a predictable developmental and morphological event implies genetic regulation, evidence for the genetic regulation of cell death was not revealed until the 1980s following studies on developmental mutants of the nematode *Caenorhabditis elegans* by Ellis and Horvitz (15). Genetic studies of the nematode identified two genes, ced-3 and ced-4, which were required for normal developmental cell death, and a third, ced-9, which appeared to act as a negative regulator of cell death (Fig. 2). The discovery of mammalian homologues of these genes initiated an intense search for new genes involved in the regulation or execution of cell death pathways. Many new cell death regulators have been identified, and a number of regulators have been shown to be previously identified oncogenes or supressor genes (e.g., *bcl-2, myc, ras*, and *p53*). Thus, the genetic regulation of apoptosis is controlled by the activation of genes whose actions are to kill the cell and the corresponding deactivation of genes whose actions are to maintain cell homeostasis (Table 1).

However, the genetic regulation of a death program can be modulated by exogenous stimuli from the cells immediate environment. The concept that certain mammalian cells are

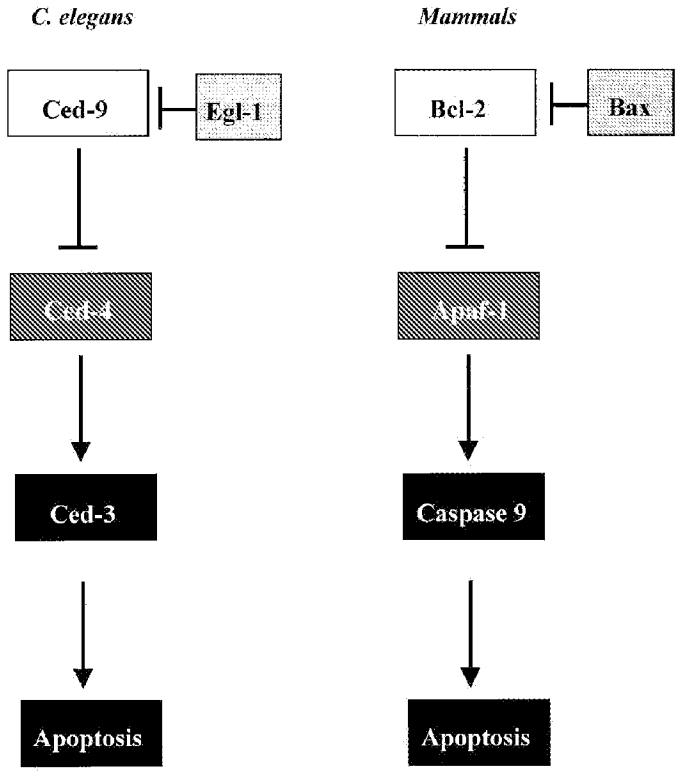

Fig. 2. **Homology between cell death pathways in *C. elegans* and mammals.** The ced 9/bcl-2 family consists of pro-apoptotic (egl-1 and bax) and anti-apoptotic (ced-9 and bcl-2) protein members. The ced-9/bcl-2 family integrates positive and negative apoptotic signals and arbitrates whether apoptosis should occur; activation of ced-4/apaf-1 commits a cell to apoptosis and the ced3/caspase family mediates the proteolytic destruction of the cell. Adapted from Adams and Cory, 1998.

TABLE 1. **Positive and negative genetic regulators of apoptosis**

Positive genetic regulators (inducers of apoptosis)	Negative genetic regulators (inhibitors of apoptosis)
c-myc	bcl-2
p53	ras
death factor and receptor expression	abl
caspases	bcl-w
bax	brag-1
bak	bfl-1
bad	bcl-x_L

under social controls with extrinsic cellular events regulating endogenous apoptotic programmes was first expounded by Raff (16): individual cells are programmed to commit suicide unless they receive signals for survival. In the presence of a limited supply of extracellular growth factors, cell numbers are maintained relatively constant as a result of competition for growth factors, thereby maintaining a balance between division and cell death. A review of the important positive and negative genetic and environmental regulators of apoptosis follows.

Positive genetic regulators

c-Myc gene family—The *myc* family of protooncogenes (Myc, Mad, Max, and Mxi-1) encode short-lived nuclear proteins with DNA-binding properties, which can heterodimerize to from transcriptional activators or repressors (17). *c-myc* is a 'Janus gene' involved in both cell proliferation and apoptosis (18). This apparent contradiction is reconciled through an understanding of the different responses exacted by survival signals from this gene. In the presence of a survival signal such as anti-apoptotic cytokines [e.g., insulin-like growth-factor-1 (19)] or overexpression of a negative regulator of apoptosis [e.g., *bcl-2*, (20)], Myc drives proliferation, and in its absence, the default program induced by Myc results in apoptosis. It is now generally believed that oncoprotein-induced apoptosis may reflect the fact that the pathways mediating growth and apoptosis are coupled processes: the dual signal model (21, 22). In this model, activation of cell proliferation necessarily primes the apoptotic program that, unless countermanded by appropriate survival signals, automatically removes the affected cell. Survival signals are normally provided by neighbouring cells, and this ensures that somatic cells remain mutually interdependent for survival and so limits the possible proliferative autonomy of any individual cell. This has direct implications for malignant progression, as generally two or more mutations are required to initiate and promote cellular transformation. Thus, the combination of deregulated Myc and survival signals promotes cell proliferation in the absence of apoptosis and provides a rationale for oncogene cooperation in tumorogenesis (Fig. 3) (23).

p53 tumor supressor gene—The p53 protein is a transcription transactivator that plays a central role in mediating the cellular response to DNA damage, helping to maintain genomic stability (24). Inactivation or loss of p53 are the most common aberrations in human cancers and they indicate that inactivation of tumor supressor genes is as equally important as activation of oncogenes like *c-myc* in tumorogenesis. Following sublethal DNA damage, p53 directs a G_1 cell cycle arrest,

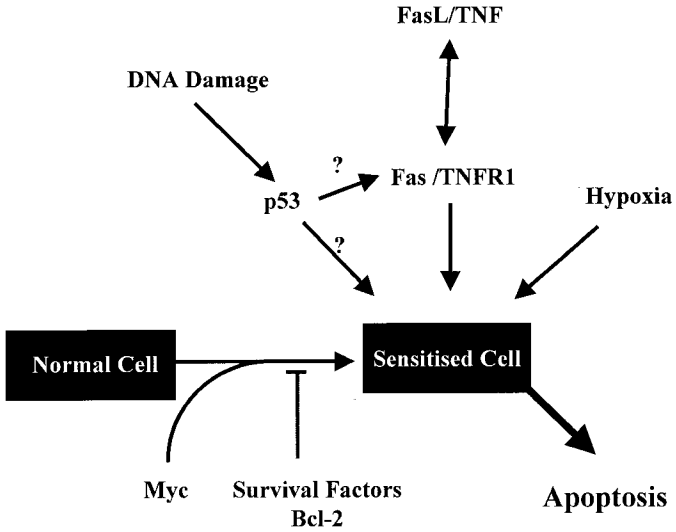

FIG. 3. **Model of the relationship between oncogenes and death signals.** In this model, oncoproteins do not trigger apoptosis directly, but they act as a sensitizer to apoptotic triggers (death receptor activation/hypoxia, etc.). In the absence of survival signals, myc sensitises the cell to an apoptotic trigger; however, the combination of deregulated Myc and survival signals act to promote cell proliferation. P53 may sensitize cells in part through upregulation of Fas, although other mechanisms likely exist. Adapted from Evan and Littlewood, 1998.

allowing DNA repair to occur prior to further replication (reviewed in Ref. 25). In the event of excessive DNA damage, p53 initiates execution of the apoptotic program (26). Although the mechanism whereby p53 induces apoptosis is controversial, several studies have suggested that p53 regulates apoptosis by transcriptional suppression of anti-apoptotic proteins such as bcl-2 and induction of proapoptotic proteins such as bax, insulin-like growth factor binding protein 3 (IGF-BP3), and upregulation of the Fas receptor (Fig. 3). However, apoptosis can proceed by p53-independent pathways (e.g., glucocorticoid-mediated apoptosis of thymocytes), and is not required for developmental cell death (27, 28). In sepsis, both p53-dependent and -independent pathways of apoptotic cell death have been reported (29). Overall, these findings suggest that the main role of p53 may be as a sensor of DNA damage and the mediation of the appropriate cellular response, cell cycle arrest, or apoptosis.

Death receptor and death factor expression—Death receptors belong to the tumor necrosis factor receptor (TNFR) gene superfamily, which is defined by similar cysteine-rich extracellular domains (30). A number of mammalian death receptors belonging to the TNFR family have been identified, including Fas, TNFR1, DR-3 (death receptor-3), DR-4, DR-5, and cytopathic avian leukosis-sarcoma virus receptor 1 (CAR1). In addition, this subfamily of TNFR contains a homologous cytoplasmic 80-amino acid domain termed the 'death domain' (DD). DDs enable death receptors to engage the cell's apoptotic machinery (31). Aggregation of these receptors by a trimeric ligand induces apoptosis by recruiting adaptor proteins. The adaptor proteins also contain a DD that interacts with the DD of the receptor. The ligands that activate the death receptors are structurally related molecules belonging to the TNF gene superfamily (30). Fas ligand binds to Fas; TNF and lymphotoxin á bind to TNFR1; Apo3 ligand (Apo3L, also called TWEAK) binds DR-3; and TRAIL (also called

Apo2 ligand) binds to DR-4 and DR-5. The ligand for CAR1 is unknown. Following ligand-receptor binding, further protein-protein interactions are involved in the signalling of the death pathway. Homotypic domain interactions between proteins is a common theme in apoptosis.

The recent identification of death factor-receptor pairs that regulate apoptosis brings a new level of complexity to the understanding of apoptotic cell death. It indicates that an external killer can control apoptosis in certain instances, and that it may trigger the death pathway through an autocrine, paracrine, or systemic fashion. Furthermore, alterations in death factor-receptor signalling may have important pathogenic roles in mediating inappropriate cell death in human diseases.

Caspase [interleukin-1β-converting enzyme (ICE)-like protease] family—Two genes were found to be essential in mediating developmental cell death in *C. elegans*: ced-3 and ced-4. The cloning and characterization of the ced-3 death-promoting gene revealed significant homology to the mammalian ICE, and provided the first indication that proteases may play a central role in apoptosis (Fig. 2) (32). Alnemri et al. (33) proposed a "caspase" nomenclature (for cysteine proteases that cleave after aspartate residues) for human members of this family (Table 2). Further support for a central role of caspases as effectors of apoptosis came from the fact that overexpression of caspases induced apoptosis (34), and the ability of selective caspase inhibitors such as viral cowpox-encoded protein CrmA prevented apoptosis (35). In sepsis, caspase inhibitors have been shown to improve survival by preventing lymphocyte apoptosis, leading to enhanced immunity (36). However, caspase-1-null mice display an apparently normal phenotype (37), suggesting that there may be functional redundancy in the caspase system, allowing a fail-safe mechanism through which the apoptotic process can be completed. However, evidence is now emerging that suggests that although caspase inhibitors may prevent certain characteristic biochemical and morphological features of apoptosis, cells that have sustained a cytotoxic insult and have been treated with caspase inhibitors have lost their replicative or clonogenic potential and all are destined to die, albeit by a slower mechanism not readily identifiable as classical apoptosis (38).

Negative genetic regulators

Bcl-2 gene family—The intrinsic susceptibility of a cell to undergo apoptosis is determined by members of the protooncogene bcl-2 gene family, the mammalian homologue of ced-9 (Fig. 2). The prototypic regulator of cell death is bcl-2. Bcl-2 sets the basic apoptotic resistance threshold of cells (39), and overexpression of bcl-2 has been shown to prolong cellular survival by blocking apoptosis induced by a broad range of signals, including ultraviolet irradiation, cytokines, growth factor deprivation, and heat shock (reviewed in Ref. 40). In addition, overexpression of the bcl-2 gene has been shown to improve survival in sepsis, with a decrease bcl-2 expression found in peripheral monocytes in patients not surviving a septic insult (41, 42).

Bcl-2 belongs to a growing family of apoptosis regulatory gene products. Several homologues of the bcl-2 gene family have been recognised, including apoptotic antagonists (bcl-2, bcl-w, bcl-x_L, bf1-1, brag-1, mcl-1, and A1) and apoptotic agonists [bax (bcl-2-associated protein x), bak, bik, bad, bcl-x_s, bid, and hrk] (43). Many members of the bcl-2 protein family are capable of directly interacting with each other through a network of homo- and heterodimers. A dynamic equilibrium is established, with the ratio of death antagonists to agonists determining a cell's life or death response to an apoptotic stimulus (44). Just how bcl-2 blocks cell death is incompletely understood. Bcl-2 appears to have a number of functions in modulating the cellular response to an apoptotic stimulus, including acting as an ion channel, a mitochondrial membrane stabilizer, and as an adaptor or docking protein (Fig. 4) (43, 45).

Ras gene family—Ras gene family members (*Ha, Ki,* and *N-ras*) encode an almost identical 21-kD membrane-associated GTP-binding protein that has been associated with both proliferation and apoptosis (46). Ras proteins are key transducers of mitogenic signals, a fact attested by the high frequency (approximately 30%) of Ras-activating mutations in human malignancies (46). Ras proteins mediate their oncogenic potential through activation of the Raf-MAP kinase pathway, promoting cells to move through G_1 of the cell cycle towards the S phase (47). In addition to inducing cellular transforma-

TABLE 2. **The human caspase family**

Caspase designation	Alternate names	Recognition site	Substrates
Caspase 1	ICE, CED-3	YVAD ↓ G YVPD ↓ S	Pro-IL1β, pro-caspase 1, 2, 3, and 4, actin, PITSLRE, PARP
Caspase 4	TX, ICH-2, ICErel-II		Pro-caspase 1 and 4, PARP
Caspase 5	TY, ICH-3, ICErel-III		
Caspase 2	ICH-1, NEDD-2		PARP, Pro-caspase 2
Caspase 9	ICE-LAP6, Mch6		PARP
Caspase 3	CPP32, Yama, apopain	DEVD ↓ G DMQD ↓ N YVPD ↓ S	PARP, DNA-PK, SRE/BP, rho-GD1 PKCδ, Pro-Rb, Pro-caspase 3
Caspase 6	Mch2	VEID ↓ NG	Lamin A, PARP
Caspase 7	Mch3, ICE-LAP3, CMH-1		PARP, Procaspase 6
Caspase 8	FLICE, MACH, Mch5		Pro-caspase 1-like, Pro-caspase 3-like
Caspase 10	FLICE2, Mch4		PARP, Pro-caspase 1-like, Pro-caspase 3-like

The caspase family can be divided into three subfamilies based on the sequence homology: caspase 1 (ICE), caspase 2 (ICH-1), and caspase 3 (CPP32), according to Alnemri et al., 1996. The Arabic numeral is assigned based on its date of publication. Pertinent characteristics, such as their alternative names, cleavage sites (if known),and substrates are also provided.

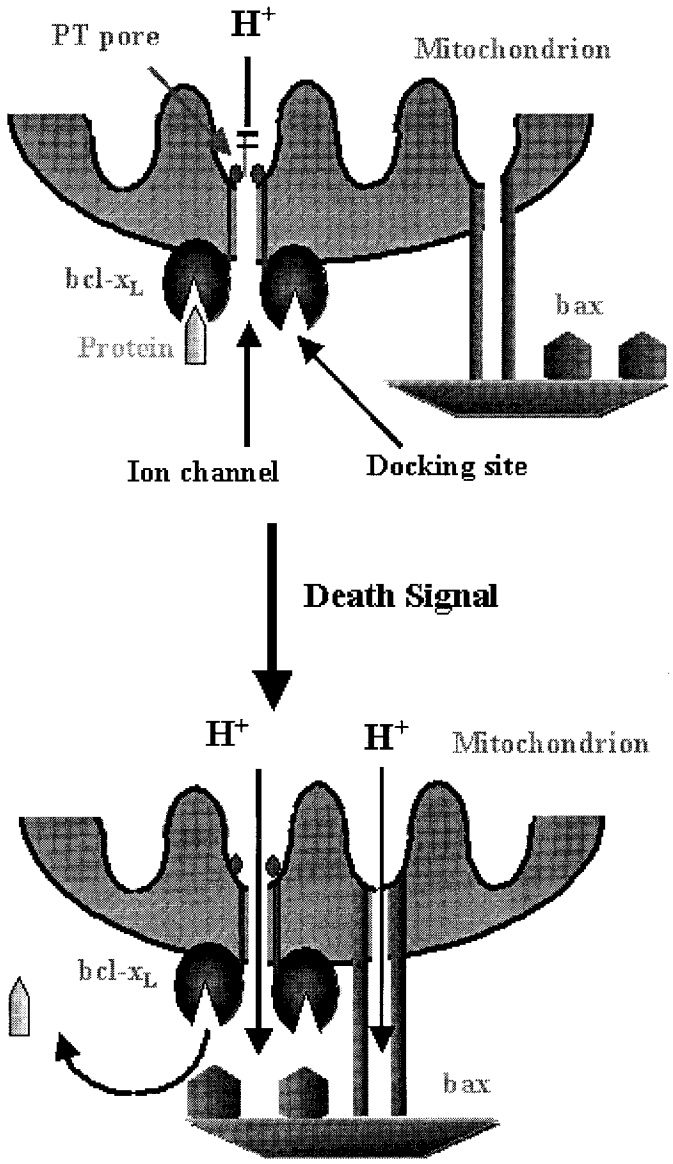

FIG. 4. **A model of the putative mechanisms of action of the bcl-2 family in regulating apoptosis.** Anti-apoptotic bcl-2 family members such as bcl-x_L appear to function at multiple levels to block apoptosis. bcl-x_L may form discrete ion channels and regulate transmembrane ion fluxes; bcl-x_L appears to act as an adaptor or docking protein by pulling other apoptotic regulating proteins out of the cytosol, functioning to either inactivate them to allow them to interact with other sequestered proteins; bcl-x_L may act as a mitochondrial membrane stabilzer by inhibiting the opening of the permeability transition (PT) pore and preventing loss of the mitochondrial membrane potential ($\Delta\psi m$). A death signal may, for example, result in heterodimerizing of pro-apoptotic bcl-2 family members such as bax with bcl-x_L and block its anti-apoptotic function. Bax may act to either block or alter bcl-x_L's ion channel function, to prevent its adaptor function, to open the PT pore, or all three. In addition, bax appears to have intrinsic ion channel activity and may fulfil its pro-apoptotic role by promoting the loss of ($\Delta\psi m$).

tion, overexpression of *Ha-ras* has been shown to inhibit apoptosis (48).

Abl tyrosine kinases—The *c-abl* protooncogene is a protein tyrosine kinase thought to play a role during cell cycle progression (49). The oncogenic forms of the *abl* family of tyrosine kinases, *bcr-abl* and *v-abl*, have been reported to mediate some of their tumorogenic effects through suppression of apoptosis (50, 51). Reciprocal translocation between *c-abl* on chromosome 9 to *bcr* on chromosome 22 produces the chimeric *bcr-abl* oncogene and a shortened version of chromosome 22, the Philadelphia chromosome, a cytological hallmark of chronic myeloid leukaemia. Upregulated *abl* kinase activity by *bcr-abl* and *v-abl* can rescue growth factor-dependent hematopoietic cell lines from apoptosis induced by growth factor withdrawal (50, 51). Recently, c-abl tyrosine kinase has been shown to be involved in lipopolysaccharide-induced macrophage activation (52).

ENVIRONMENTAL REGULATORS

In addition to intrinsic genetic factors, extracellular factors also regulate the susceptibility of cells to undergo apoptosis. Many soluble circulating factors, previously referred to as growth factors (e.g., insulin-like growth factor and platelet-derived growth factor), are capable of delaying apoptosis and maintaining cell viability in the absence of proliferation. Such factors are now referred to as 'survival factors.' Raff's 'social control hypothesis' of population size regulation has gained increasing support over the last number of years and proposes that all cells (with the exception perhaps of the blastomere) express a default apoptotic program and will undergo apoptosis unless they are rescued by the presence of survival factors (16). This hypothesis suggests that specific cell types would be confined to the tissues producing their survival factor, and availability of the survival factor would limit population size. Thus, removal of survival factors may be a common pathway to apoptosis.

For example, hematopoietic progenitor cells and differentiated cells have been shown to be dependent on specific survival factors such as granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), IL-3, and erythropoietin (reviewed in Ref. 53). The requirement of immune cells for survival signals may be fundamental in ensuring appropriate expansion and retraction of immune cells during and after an immune response. Following a systemic insult, various proinflammatory cytokines such as IL-1β, TNF-α, and GM-CSF are released both locally and systemically, and, at concentrations found *in vivo*, have been shown to delay monocyte and neutrophil apoptosis *in vitro* (54, 55). Monocytes and neutrophils activated in the inflammatory response have also been shown to produce IL-1β and TNF-α (54, 55)), suggesting that both autocrine and paracrine mechanisms may influence cell survival. Following resolution of inflammation, a local decrease in cytokines could then be sufficient to induce apoptosis in the expanded proinflammatory cell population with a return to homeostasis.

CELLULAR SIGNALLING DURING APOPTOSIS

Given the extensive regulatory controls evident in apoptosis, it is perhaps not surprising that a similarly complex and diverse range of intracellular signalling molecules are generated following initiation of an apoptotic response. Various molecular mediators have been implicated in the apoptotic signalling cascade, including kinases, calcium, ceramide, and reactive oxygen species (ROS). The relative conservation of morphological and biochemical events observed between different

species and cell types in the terminal stages of apoptosis has prompted many investigators to postulate that these diverse signals may converge upon a central effector or executioner of the apoptotic process (56–58). The existence of a common final pathway may explain how negative regulators of apoptosis such as bcl-2 can inhibit apoptosis following initiation by a broad spectrum of insults. Several researchers have appointed the mitochondrion as the central executioner of the apoptotic process (56, 57) acting to orchestrate the final degradative phase of apoptosis. Accordingly, the apoptotic process can be arbitrarily divided into several overlapping but conceptually distinct phases: the induction phase in which the apoptotic process is triggered and common intracellular mediators are released; the central execution or effector phase in which the diverse pro- and anti-apoptotic signals are coordinated; and the final degradative stage is triggered in which the characteristic morphological and biochemical features of apoptosis become evident. The division of apoptosis, a continuous, complex process with multiple feedback regulatory loops, into arbitrary phases, although highly theoretical, does allow us to discuss, in a rational manner, important signalling events in the apoptotic cascade.

Induction and common signals in apoptosis

Death receptor signalling for apoptosis—In the last number of years there has been an exponential increase in our understanding of the initial death effector and regulator proteins involved in coordinating the release of apoptotic mediators initiated following ligation of the death receptor by its ligand. This knowledge has provided us with tantalizing glimpses at the complexity and hierarchy of the death signal transduction pathway. What is now clear is that there exists multiple signalling pathways to cell death. Which pathway, or pathways, employed depends on the initial death signal triggered, the cell type, and the balance between apoptotic and survival signals. As in the case of growth regulation, multiple signalling pathways allow for multiple checkpoints and fine-tuned regulation of apoptosis. Fas- and TNF-mediated apoptotic pathways are the most widely investigated and Figure 5 addresses the Fas-FasL interaction. Briefly, binding of FasL to Fas induces trimerization of the Fas receptor and activation of its cytoplasmic death domain, recruiting a set of intracellular proteins into a death-inducing signalling complex (59). Three Fas-mediated apoptotic pathways have been proposed (Fig. 5). The best characterized pathway involves the adaptor protein Fas-associated death domain (FADD), which interacts with Fas via its c-terminal death domain and recruits pro-caspase 8 via its n-terminal death effector domain, inducing activation of the protease domain of caspase 8 (FLICE) (60). Caspase 8 activates downstream effector caspases such as caspase 9, the mammalian functional homologue of ced-3, committing the cell to apoptosis.

Kinases—Following initiation of the apoptotic program, a series of signalling mediators are activated, which include early activation of protein tyrosine kinases (PTK). These enzymes can result in a cascade of signalling events, including activation of phosphatidylinositol 3-kinases, phospholipases, phosphatases, and downstream changes, including calcium mobilization, activation of protein kinase C (PKC), MAPK, and activation of transcription factors (61). The effect of PTK signalling in apoptosis is dependent both upon cell type and on the activation state of the cell. Activation of PTK has been shown during apoptosis of peripheral lymphocytes (62), suggesting that tyrosine phosphorylation of target proteins positively regulates apoptosis in these cells. In contrast, activation of PTK in neutrophils (63) and eosinophils (64) prevents apoptosis and has been observed during sepsis (54).

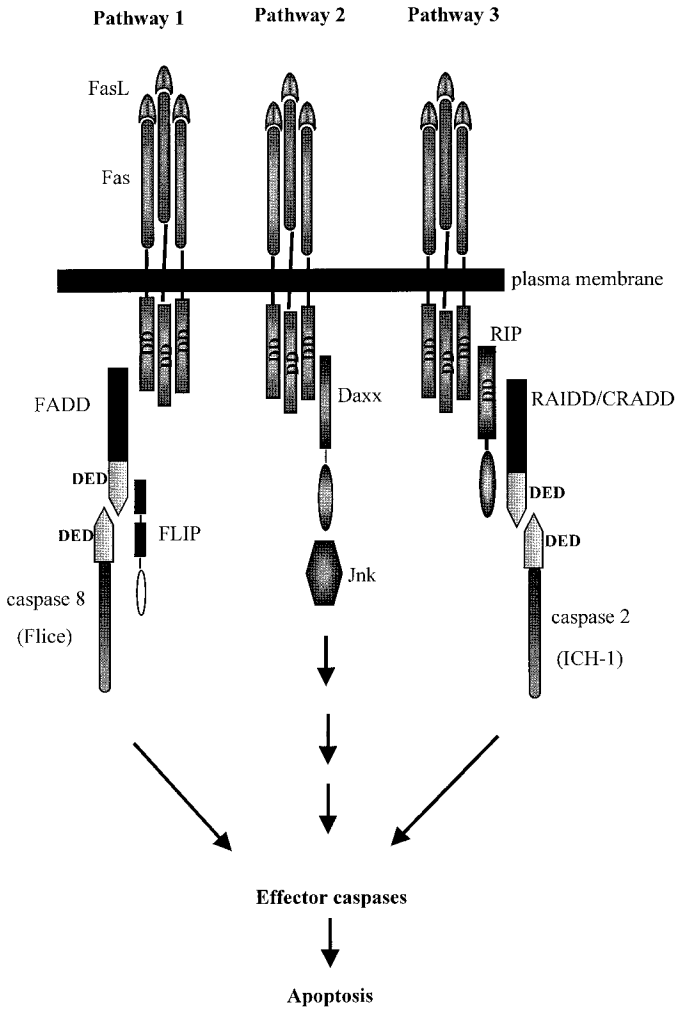

FIG. 5. **Fas apoptotic pathways.** Pathway 1 involves Fas-FADD-caspase 8 interactions. Pathway 2 is transduced by Fas-Daxx-Jnk. Pathway 3 involves Fas-RIP-RAIDD-caspase 2 recruitment. FLIP associates with FADD to form a stable Fas-FADD-FLIP complex that inhibits caspase 8.

The effect of PKC on apoptosis is dependent upon a number of factors, including the specific cell type involved (65), the activation and functioning of intracellular phosphatases (64), and the isoforms of PKC activated (66). Recently, inactivation of PKC-α has been shown to be involved in polymicrobrial sepsis (67).

Calcium—A consistent event in apoptosis in many cell systems is the progressive influx of calcium ions, resulting in a sustained elevation in intracellular free calcium. Calcium ions appear to play a central role in mediating apoptosis in certain cell types, with removal of extracellular calcium or buffering intracellular calcium effectively inhibiting apoptosis in these cells (2, 68). Calcium has been shown to have multiple

potential sites of action in the apoptotic process, including activation of enzymes such as endonuclease, tissue transglutaminase, and proteases, chromatin organization, and gene regulation. However, in the majority of cases, alteration in cellular calcium levels does not appear to be essential for the induction of apoptosis, raising the possibility that alterations in calcium concentrations in certain cells occur as a consequence of apoptosis. Furthermore, apoptotic cell death following calcium influx is not universal; indeed, elevation of intracellular calcium has been shown to delay apoptosis in certain cells such as neutrophils (69).

Cellular redox state—Alteration in redox potential favoring a pro-oxidative state has been observed within many cell systems following the induction of apoptosis by a diverse range of stimuli and has been proposed as a major apoptotic mediator (58). However, the requirement for ROS production is not absolute as ROS production is not evident during induction of apoptosis in all cell systems. Recent reports indicate that the redox state of the cell may be altered during apoptosis without antecedent generation of ROS through a mechanism involving intracellular glutathione depletion (70). In addition, proapoptotic stimuli can induce apoptosis in the absence, or near absence, of oxygen, which implies that ROS are not the *sine qua non* of apoptosis (71).

Redox-sensitive targets important in the mediating intracellular messenger cascades in apoptosis have been identified. Both ROS production and/or antioxidant disruption have been shown to activate tyrosine kinases (72) and induce calcium release (73) and, recently, proteolytic cleavage of the caspase substrate PARP, suggesting that oxidative targeting may play a role in activation of caspases (74). These studies strengthen the view that oxidative stress is a major, but not exclusive, mediator in the apoptotic pathway.

THE MITOCHONDRION: A CENTRAL EFFECTOR?

The ability of multiple signalling cascades to engage what appears to be a common degradative pathway in apoptosis suggests that a central effector must link and orchestrate a diverse range of signals to the activation of downstream terminal events in apoptosis. Several groups have designated the mitochondrion as this putative central effector of the apoptotic programme (56, 57, 75). Mitochondrial intermembrane proteins, cytochrome c, and a protease called apoptosis-inducing factor are released during apoptosis and are important effectors of the apoptotic process (76, 77). The discovery of the mammalian homologue of ced-4, designated Apaf-1 (apoptosis activating factor-1), has provided suggestions of how mitochondrial regulation of apoptosis is achieved (78). Apaf-1 is a 130-kD protein with a COOH-terminal region comprising multiple tryptophan-aspartate repeats, which are proposed to mediate protein-protein interaction. Apaf-1, cytochrome c (Apaf-2), caspase-9 (Apaf-3), and dATP are sufficient to activate pro-caspase-3 (78, 79) (Fig. 6). Caspase-9 appears to be the most upstream member of the apoptotic cascade triggered by cytochrome c and dATP, and results in cleavage and activation of caspase-3 (79). Recently, bcl-x_L has been shown to physically interact with caspase 9 and Apaf-1 to form a ternary complex, termed an apoptosome, with bcl-x_L inhibiting the maturation of caspase 9 mediated by Apaf-1 (80). These results provide important insight into the roles of Apaf-1, cytochrome c, and bcl-2 family members in the regulatory control of caspase activation (75).

Despite the growing evidence supporting the central role of mitochondria in regulation and activation of caspases, recent reports have cast doubt on the absolute universal requirement of mitochondria in apoptosis. These reports reveal activation of caspases by Fas, ceramide, and staurosporine prior to loss of mitochondrial transmembrane potential (81), activation of caspase 8 at the Fas receptor level (59), and the fact that certain cell lines that lack mitochondrial DNA and therefore do not have a functional respiratory chain can still be induced to die by apoptosis (82). These studies suggest that the role of the mitochondrion as a central executioner may not apply in all cell systems, and that there exists an element of redundancy in the apoptotic program: by inhibiting one death pathway, another pathway may be able to take its place, allowing cell apoptosis to proceed as normal. This raises the possibility that there may not, in fact, be a common mediator in all apoptotic pathways.

SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) AND MULTIPLE ORGAN DYSFUNCTION SYNDROME (MODS)

The theory that apoptosis contributes to the multiple organ dysfunction of sepsis was formulated originally by Bone in 1996 (83). The argument put forth postulated that certain immunomodulatory factors present in overwhelming amounts

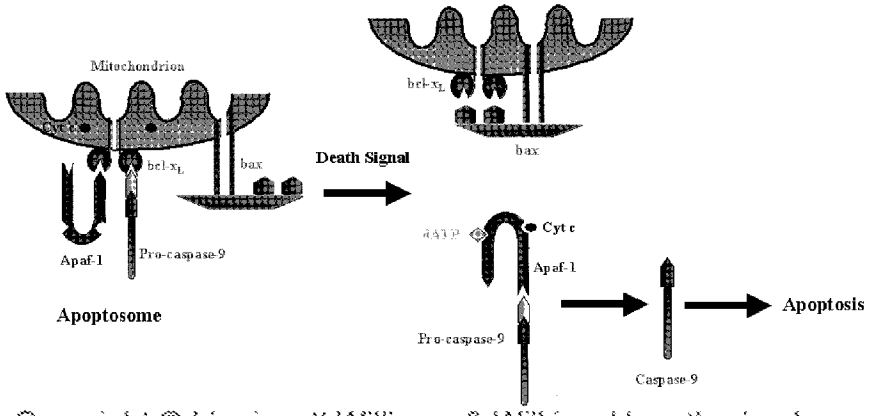

FIG. 6. **A model of Apaf-1 regulation by the bcl-2 family.** bcl-x_L binds Apaf-1 and pro-caspase 9, forming a ternary complex termed an apoptosome, and prevents maturation of pro-caspase 9 by Apaf-1. A death signal may, for example, promote interaction of bax (or another pro-apoptotic bcl-2 family member) with bcl-x_L, preventing it from neutralizing Apaf-1. Apaf-1, in conjunction with cytochrome c released from the mitochondrion and dATP, can then activate pro-caspase 9. Caspase 9 subsequently activates effector caspases, resulting in apoptosis.

in SIRS could contribute to a generalized systemic increase in cellular apoptosis, thereby accounting for the organ failure of MODS. As SIRS is essentially an imbalance between pro- and anti-inflammatory immune activity, it follows that the induction (or even inhibition) of apoptosis occurring in SIRS is inappropriate and ultimately more autotoxic than beneficial. Certain specific pathophysiologic conditions inextricably linked to SIRS and the onset of MODS have been shown to differentially modulate apoptotic rates in organ tissue cells and their respective endothelial infrastructures (84). These include increased proinflammatory [TNF, interleukin 1β, interferon-γ (IFN-γ), interleukin 8, etc.] and anti-inflammatory (interleukin 6, interleukin 10, etc.) cytokines, elevated glucocorticoid levels secondary to adrenal cortex stimulation, increased production of ROS associated with ischemia/reperfusion, and the presence of bacterial wall products in the systemic circulation.

Increased proinflammatory and anti-inflammatory cytokines

TNFα is one of the major cytokines in the cytokine cascade observed in SIRS. As a major pro-apoptotic mediator, it has been implicated in the increased apoptosis seen in a variety of tissue types that become dysfunctional during SIRS. It is capable of inducing apoptosis in endothelial cells (85), hepatocytes (86), and thymocytes (87). The half-life of circulating TNF is 14 to 18 min in humans. In healthy subjects, plasma levels rarely exceed 35 pg/mL (88), and at slightly higher concentrations, it plays a role in cell proliferation and differentiation and regulation of cytokine interaction (89, 90). Therefore, at these levels, TNF induces mechanisms for tissue remodelling, inflammation, and cytotoxicity. However, these biologic effects of TNF are elicited when only 5% to 10% of TNF receptors on cells are occupied, and it is for this reason that when TNF exists in overwhelming amounts, as occurs in SIRS, we observe widespread tissue injury and dysfunction, in part related to increased apoptosis. Furthermore, the apoptotic effects of TNFα may be augmented by IFN-γ (91) and mediated by nitric oxide (NO) (92), both of which exist in greater quantities in SIRS.

Interleukin-1β is a cytokine with potent proinflammatory capabilities. Il-1 attends in two forms, Il-1α and Il-1β, produced by macrophages, monocytes, and to lesser degrees by other cell types. Both are recognized equally by one Il-1 receptor, but Il-1β is the predominant form *in vivo*. In healthy humans, levels of Il-1β remain below 60 pg/mL with a half-life of 6 to 10 min. Intravenous murine administration of 10 to 100 ng/mL results in fever, neutrophilia, and increased acute-phase protein synthesis (93). Higher levels induce hypotension, leukopenia, tissue injury, and death, i.e., a SIRS/MODS-like state. These effects are not as profound as those seen with TNF administration, but Il-1 accounts for significant neutrophil margination, leukocyte binding, and procoagulant activity (94). The proinflammatory effects of TNF and Il-1 are cumulative and each stimulates the production of the other, indicating that they probably account for the majority of the systemic effects typical of SIRS. As is the case for TNF, animal studies employing blockade of Il-1 activity with receptor antagonists have demonstrated improved survival in gram-negative bacterial sepsis (95). Il-1β is associated with pancreatic cell apoptosis (96, 97) and an increase in constitutive NO production. This NO-regulated mechanism appears to be responsible for Il-1β-induced chondrocyte apoptosis, as well (98). Although Il-1β has similarly been reported to excite leukemic cell apoptosis (99), it mediates significant retardation/inhibition of neutrophil apoptosis (100). Inhibition of neutrophil (PMN) apoptosis is associated with SIRS/MODS, and this may be signalled for by a variety of other pro- and anti-inflammatory cytokines such as GM-CSF, interleukin 8, interleukin 6, and a number of members of the CXC chemokine family (101–103). The anti-inflammatory cytokine Il-10 has recently been shown to reverse the anti-apoptotic effect of various proinflammatory mediators such as LPS, IFN-γ, and GM-CSF (104).

Glucocorticoid-mediated cellular apoptosis

The stress response associated with SIRS/MODS results in elevated levels of circulating glucocorticoids such as cortisol via adrenocorticotrophic hormone stimulation of the adrenal cortex. Glucocorticoids have been reported to induce apoptosis in lymphocytes (105) and immature thymocytes (106), possibly through the glucocorticoid receptor (107). An unsurprising finding in view of the differential effects already observed in neutrophil/PMN programmed cell death is that glucocorticoids prevent neutrophil apoptosis (108), and this phenomenon is closely linked with increased superoxide anion production by these self-same cells (109).

ROS and apoptosis

ROS are widely generated in biological systems, and elevated levels are a characteristic of SIRS/MODS. Oxidative stress in response to various external stimuli has been implicated in the activation of transcription factors and the induction of apoptosis. Oxygen free radicals induce DNA sequence changes in the form of mutations, deletions, gene amplification, and rearrangements that may trigger apoptotic cell death. ROS play a pivotal role in the programmed cell death occurring in many mammalian cell types, including hepatocytes (110), vascular smooth muscle cells (111), primary thyroid cells (112), HL-60 cells (113), endothelial cells (114), PC12 cells (115), and mesangial cells (116) amongst others. Oxidants such as H_2O_2, O_2^-, and OH^- are the primary ROS involved, their mechanisms dependent to a large degree on intracellular pathways recruiting PKC, etc. Certain cytokines (GM-CSF, etc.) have been shown to delay PMN apoptosis through inhibition of spontaneous ROS generation (117). NO is a reactive radical that is also elevated after trauma and sepsis (118) and is induced by LPS (119). NO has been implicated in apoptosis induction in numerous cell types, including thymocytes, neutrophils, and endothelial cells (120), and may also be involved in TNF-mediated apoptosis (121).

Bacterial wall products and apoptosis

Bacterial wall products remain the most frequently encountered (in the clinical setting) and most potent of exogenous mediators of inflammation. Pre-eminent amongst these is lipopolysaccharide (LPS/endotoxin), a constituent of the outer wall of gram-negative bacteria. Myriad animal studies have demonstrated that administration of LPS, either intravenously or

intraperitoneally, results in a clinical syndrome mimicking SIRS/MODS, which typically results in shock and death. Structurally, LPS consists of a highly conserved active lipid A moiety, a variable core oligosaccharide region, and an O side chain, all which collectively exist in the outer membrane of gram-negative bacteria. It interacts with a variety of cell types via the receptors CD14, CD11/18, and Toll-like receptors 2 and 4. Utilizing CD14 to stimulate cells requires the presence of LPS-binding protein in serum. This enhances cellular responses to LPS and is an innate protective device designed to activate the immune system even in the presence of minute quantities of endotoxin. CD14 also exists in serum and participates in all phases of LPS-induced SIRS due to its ability to induce LPS sensitivity in cells lacking CD14 receptors (e.g., endothelial cells). LPS induces profound TNF and Il-1 release from macrophages via activation of p38, a 38-kD member of the mitogen-activated protein (MAP) kinase family (122). It has emerged that a number of gram-negative and gram-positive bacterial products can initiate a SIRS-like state in animal models and have LPS-like effects in *in vitro* experiments, suggesting that LPS is just one of many molecules that mediates bacterial sepsis.

The gut hypothesis of SIRS has been used to explain why no identifiable focus of infection can be found in as many as 30% of bacteremic patients who die from SIRS/MODS. Clinical evidence abounds demonstrating that intestinal permeability is increased in patients with SIRS. This facilitates translocation of bacteria colonizing the intestinal tract into the mesenteric lymphatics from where they gain systemic access and mediate end-organ damage. Modulation of cellular apoptotic rates is one mechanism by which bacterial wall products effect this end-organ damage.

In vitro evidence that LPS influences apoptosis—"Shock lung" or Adult Respiratory Distress Syndrome (ARDS) is a hallmark of SIRS/MODS, and is associated with gram-negative bacterial sepsis. This may in part be related to decreased efficacy of the resident population of alveolar macrophages. The lung injury associated with SIRS is predominantly mediated by neutrophils. Compromised removal of these PMNs by alveolar macrophages may allow neutrophils and their proinflammatory mediators to persist within the lung parenchyma, allowing propagation of tissue damage. LPS has been shown to increase apoptosis in alveolar macrophages by as much as 4-fold (123). This assumes profound significance when applied to the natural history of SIRS, as the most potent endogenous regulators of SIRS/MODS (Il-1β, TNFα, Il-6, GM-CSF, IFN-γ etc.) are incapable of enhancing alveolar macrophage apoptosis (123). In support of this theoretical framework, it has been reported that LPS delays PMN apoptosis (100), which may account for the increased PMN inflammatory infiltrate and sequestration associated with ARDS in the presence of sepsis. Other investigators have shown that LPS is also capable of signalling cell death in macrophages of peritoneal residency (124), and that this apoptosis was dependent on inducible NO synthase (iNOS), an enzyme programming for one of the oxidants mentioned previously. Renal failure occurring in the presence of gram-negative sepsis has similarly been linked to renal cell apoptosis. LPS has been shown to increase levels of Fas mRNA in a time- and dose-dependent manner in renal mesangial and tubular cells. An overall increase in Fas mRNA and protein within the kidney itself has also been demonstrated with resultant increased apoptosis along nephron units (125). Messmer et al. (126) have demonstrated that LPS elicited glomerular endothelial cell (EC) apoptosis in a time- and concentration-dependent fashion and it was more rapidly induced than similar changes associated with TNFα. Furthermore, LPS had a more pronounced effect than TNFα on expression of the pro-apoptotic protein *Bak* and the anti-apoptotic *Bcl-xl* protein (126). Despite these findings, the effect of LPS on EC apoptosis remains controversial, with many investigators reporting divergent results. Frey and Finlay (127) contest that LPS-mediated apoptosis in a bovine EC line is a soluble CD14-dependent phenomenon, as measured using DNA fragmentation assays, confocal/transmission electron microscopy, and anti-CD14 monoclonal antibodies. This induction of EC apoptosis by LPS is supported by work from other researchers who have identified that LPS-mediated EC apoptosis occurs via a FADD-dependent pathway that is independent of TNFR1 and Fas, suggesting that LPS may utilize a novel DD-containing protein to transduce the death signal, at least in EC (128). Evidence that LPS does not accelerate EC apoptosis *in vitro* accrues from a variety of sources (129, 130). At least two cytoprotective proteins are induced by LPS in cultured human EC. LPS upregulates expression of the Bcl-2 homologue, A1, and the zinc-finger protein A20 in microvascular EC. Induction of these proteins is once again dependent on CD14 and requires activation of NFκB (129). Eissner et al. (130) have shown that LPS alone is unable to induce programmed cell death in EC, but may augment ionizing irradiation-mediated macrovascular and microvascular EC apoptosis. Modulation of EC apoptosis by LPS remains poorly understood but is undoubtedly an area that demands in-depth investigation. As the principal effector of gram-negative sepsis, the effects of LPS on ECs must be ascertained because the systemic damage occurring in SIRS/MODS results from local loss of capillary integrity at distant sites, with subsequent mediator spillage out into end organs precipitating end-organ destruction.

As mentioned earlier, LPS is not the only bacterial wall regulator of apoptosis. Bacterial lipoproteins, which occupy similar locations in the outer wall of gram-negative bacteria, are also capable of influencing cellular apoptosis. Aliprantis et al. (131) has shown that lipoproteins initiate apoptosis in a THP-1 monocytic cell line through Toll-like receptor-2 (hTLR2) and in an epithelial cell line transfected with hTLR2, indicating a molecular link between other bacterial wall products, apoptosis, and host defense mechanisms.

In vivo evidence that LPS influences apoptosis—In vivo evidence of the modulatory effects of LPS on cellular apoptosis abounds. Much of the preliminary work on apoptosis concentrated on thymocytes, and subsequent *in vivo* research confirmed apoptosis in the thymus and other lymphoid organs in response to LPS. Zhang (132) found that administration of LPS induced DNA fragmentation in the thymus of mice, confirmed by gel electrophoresis and laser flow cytometry. This was mediated in part by adrenal hormones (e.g., gluco-

corticoids) and TNF-α as adrenalectomy and anti-TNF-α monoclonal antibody abrogated DNA fragmentation significantly. Further studies with similar methodology reproduced these findings in thymocytes, but in contradiction to Zhang, apoptosis was also detected in bone marrow and spleen (133). IFN-γ augments LPS-induced thymocyte apoptosis if mice are pretreated with IFN-γ; however, the simultaneous injection of both completely prevents thymocyte apoptosis. These findings are linked to increased levels of TNF-α and serum cortisol arising from IFN-γ pretreatment (134).

Pancreatitis has long been recognized as an event predisposing to SIRS/MODS and is associated with significant mortality often arising from organ-failure distant to the pancreas, e.g., lung or kidney. Sections of pancreata from experimental and clinical pancreatitis are characterized by a massive inflammatory PMN infiltrate (135), and it is conceivable that delayed PMN apoptosis contributes significantly to pancreatic damage in this context. It has been postulated that a subclinical LPS insult in individuals with pancreatitis of diverse etiology may be the decisive factor accounting for mortality. Fortunato (136) has suggested that prolonged alcohol consumption may sensitize pancreatic acinar cells to endotoxin-induced apoptosis. Murine studies revealed that long-term alcohol consumption suppressed apoptosis in the pancreas, but that LPS injection upregulated this process. This upregulation correlated with increased caspase-3 activity and decreased bcl-2 expression (136). Similar results were observed in cerulein-induced pancreatitis, strengthening the hypothesis that the pathological features of acute pancreatitis might be modified by the presence of non-fatal endotoxemia through the induction of acinar cell apoptosis (137).

Lung injury remains the primary event heralding the onset of widespread MODS arising from SIRS. LPS administration (20 mg/kg bodyweight) and timed sacrifice yielded histologic findings of acute lung injury (ACI) in a study designed to elucidate the role of endothelial apoptosis in LPS-mediated ACI. Further analysis demonstrated DNA fragmentation in EC, but also in bronchial and alveolar epithelial cells (138). In the face of such findings, researchers have attempted to suppress apoptosis as a therapeutic modality. Caspase inhibition with Z-VAD.fmk, before and after administration of LPS, has been shown to decrease caspase 3 activity and other apoptotic parameters within lung tissue and to ultimately increase survival in a murine model of ACI (139). The anti-inflammatory cytokine Il-10 has been shown to be protective in models of sepsis. In a model of LPS-induced ALI, Il-10 was found to significantly reduce the extent of lung neutrophilia at 18 h, and in separate experiments, this was attributed to an inhibition of LPS-induced increases in neutrophil survival occurring in a dose-dependent fashion. The investigators concluded that the mechanism of this anti-inflammatory effect may be through the prevention of LPS-stimulated PMN survival (140).

Although conventional thinking holds that LPS is largely ignored by T lymphocytes, some studies question this view. Castro et al. (141) performed a series of experiments that revealed that LPS engages most CD4 and CD8 T cells as measured using the lymphocyte activation markers, CD69 and CD25. *In vivo*, this resulted in massive T cell apoptosis over a period of days following LPS exposure (141). As T cells are a prerequisite in the mounting of an effective host immune response, these observations may contribute some pertinent information on the immunologic consequences of endotoxic shock and organ failure.

We have mentioned how bacterial translocation from the gut to the systemic circulation via the lymphatics may be a crucial step in the establishment of SIRS/MODS. This naturally presupposes that either gut mucosal epithelial layer or submucosal endothelial integrity is somehow compromised. There is substantial evidence, already cited, that endothelial function is altered by increased apoptosis, but there is increasing proof that LPS modulates epithelial cell apoptosis, perhaps promoting the likelihood of a mucosal breach. For example, LPS from *Helicobacter pylori* has been identified as a virulence factor for the induction of gastric epithelial cell apoptosis. Two days after intragastric application of 200 μg of LPS to rats, the gastric epithelial apoptotic index was recorded at 71.9%, increasing to 76.8% after 10 days (142). Findings such as these may account for the phenomenon of bacterial translocation, although there remains substantial work to be done in this area.

The preterminal event in MODS is usually severe hemodynamic instability, refractive to volume and inotropic support, associated with recondite lactic acidosis indicative of impaired perfusion and altered cellular metabolic function. The hemodynamic instability and impaired perfusion may in part arise from altered cardiac myocyte function. A recent study has identified a direct effect of LPS on rat cardiac apoptotic rates as a result of endotoxemia *in vivo*. Twenty-four hours after LPS administration (4 mg/kg), hearts were collected from treated rats, and activation of a cardiac myocyte apoptotic pathway was identified by a 1000-fold increase in caspase 3 activity with associated increases in expression of the pro-apoptotic protein bax (143). In light of such findings, it is worth considering that altered cardiac function leading to impaired perfusion may play a role in gram-negative sepsis.

Most research addressing SIRS/MODS has confirmed a pivotal role for LPS in the systemic effects observed in both gram-negative sepsis and to a lesser degree in inflammation of sterile origin, i.e., trauma and pancreatitis. There are a few studies suggesting that organ failure following endotoxemia is not related to LPS-induced apoptosis or that apoptosis following endotoxemia is independent of LPS (144, 145). However, these are in the minority, and the vast bulk of evidence clearly indicates that LPS administered *in vivo* has profound effects on cellular apoptosis and, ultimately, organ failure.

EVIDENCE THAT ALTERED APOPTOTIC RATES INFLUENCE SIRS/MODS IN HUMANS

In light of such overwhelming evidence from so many well performed studies, it can be concluded that organ tissue cell apoptosis does occur in experimental models of SIRS/MODS. Far fewer projects investigating apoptosis in active human illness have been conducted, consequently, there is a dearth of evidence confirming this phenomenon in critically ill patients. Nevertheless, what little work has been done in patients with SIRS/MODS provides compelling documentation of significant apoptotic activity occurring in critical illness.

Elevated levels of soluble Fas have been recorded in MODS patients as compared with patients without infection. The concentrations of soluble Fas were found to correlate closely with similarly raised levels of NO. Of paramount importance in this study was the decrease in the levels of these pro-apoptotic proteins in response to alleviation of MODS. The authors proposed soluble Fas levels as a potential marker of apoptosis and consequently MODS (146).

Further work investigating Fas-mediated apoptosis has been conducted in a group of patients responding to surgical stress. This study identified increased apoptosis occurring in peripheral blood mononuclear cells of patients making an uncomplicated recovery from surgery. Fas-positive lymphocytes increased significantly 2 h after surgery and remained elevated for a period of 7 days prior to returning to baseline preoperative levels. The decrease in circulating mononuclear cells postoperatively was attributed to increased apoptotic rates as confirmed by obtaining postsurgery samples and identifying apoptotic-specific chromatin condensation and DNA laddering in *in vitro* cultures after 24 h (147).

Yamada et al. (148) reported increased levels of nuclear matrix protein (NMP) in 46 patients with MODS. NMP is a general cell death index not solely specific to apoptosis; however, concentrations correlated with MODS scores and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores. These findings were made more apoptotic-specific by correlating NMP with increased levels of TNFα and NO metabolite concentrations (148).

Plasma from nine SIRS/MODS patients (29% mortality) was found to inhibit PMN apoptosis as opposed to no anti-apoptotic effect when PMNs were incubated with autologous serum (117). This study suggests that delayed PMN apoptosis in SIRS/MODS patients may in part mediate significant tissue injury propagating critical illness.

Hotchkiss et al. (149) demonstrated rapid onset of intestinal epithelial and lymphocyte apoptosis in patients with severe trauma and shock. The design involved obtaining intraoperative intestinal tissue from 10 patients with acute traumatic injury and comparing samples with intestine from six patients undergoing elective bowel surgery. Apoptosis was evaluated using light and confocal microscopy using the nuclear staining dye Hoechst 33342 and immunohistochemical staining for active caspase 3 and cytokeratin 18. They found that extensive focal crypt epithelial and lymphocyte apoptosis occurred in the majority of trauma patients, the severity of traumatic injury correlating with the degree of apoptosis. Tissue lymphocyte apoptosis was associated with a markedly decreased circulating lymphocyte population in 9 of 10 trauma patients. They concluded that apoptotic loss of intestinal epithelial cells may predispose to bacterial translocation and that increased lymphocyte apoptosis may impair immunologic defenses. In a seminal work, the same authors investigated multiple organ-specific apoptotic cell death in patients with sepsis, shock, and MODS (150). A prospective study of 20 patients who died of MODS was performed and results were compared with a control group of 16 critically ill, non-septic patients. Apoptosis was evaluated in hematoxylin and eosin-stained (H&E) specimens by deoxyuridine tri-phosphate nick end-labelling (TUNEL) and DNA gel electrophoresis. H&E-stained specimens from septic patients demonstrated apoptosis in 56.3% of spleens, 47.1% of colons, and 27.7% of ileums. There was extensive depletion of lymphocyte population in white pulp and a marked lymphocytopenia in 75% of patients with MODS, both indirect indicators of increased apoptosis. H&E staining of non-septic patient tissues revealed low rates of apoptosis. Immunohistochemical staining for active caspase 3 showed a markedly significant increase in activity in septic vs. non-septic patients. The authors state that this particular study (150) is the most exhaustive histopathologic study performed to date of patients dying from sepsis. In view of this and from our own literature review, this particular work must be the benchmark by which further studies will be measured. It represents the single greatest resource of data suggesting a critical role for altered apoptosis in MODS. However, it does not identify whether apoptosis is beneficial or detrimental to the host in these circumstances. This remains the crucial dilemma underlying the future prospects of therapeutic apoptotic modulation in the treatment of SIRS/MODS.

MODULATION OF APOPTOSIS AS IMMUNOTHERAPY

This review of apoptosis and its relationship to SIRS/MODS gathers together the bulk of academic and clinical evidence pertinent to the human condition. Teleologically, the immune systems of diverse mammals should differ in many respects, but underlying tenets, principles, and mechanisms should remain constant. However, the application of immunotherapeutic strategies gleaned from animal studies to the bedside situation is often disappointing. TNF has long been considered the predominant proinflammatory cytokine responsible at a molecular level for much of what transpires at a clinical level in SIRS/MODS. This school of thought led to much industry in the field of TNF blockade as a form of immunotherapy. Despite encouraging results from animal studies, there are contradictory data pertaining to human trials. Antibodies directed at TNF have decreased the 3-day mortality rate in humans, although there was no decrease in the rate of mortality at 28 days between treated and non-treated groups (151). A phase II and one phase III trial of anti-TNF monoclonal antibody demonstrated no difference in survival between test and control groups. Of note in the phase II trial, it transpired that patients administered medium to high doses of the antibody had less favorable outcomes than the placebo group (152).

Few clinical trials have addressed the use of anti-apoptotic agents because, quite frankly, this remains a nebulous area. Because the more downstream intracellular effectors of apoptosis (e.g., caspases) are remarkably consistent within disparate cell types, the desired effect on apoptotic rates of any single modulatory agent directed at these proteins is likely to be global. Our current understanding of the apoptotic events occurring within cells and their implications for organ function does not permit us, scientifically or ethically, to indiscriminately use agents that block apoptosis. To illustrate this point simply, we need only cite the granulocyte as an example. For the most part, this text has concerned itself with enhanced apoptosis as

a cause of organ compromise. However, enhanced neutrophil apoptosis could putatively improve the outcome in ARDS, a prequel to MODS. Hints that nature already employs such a mechanism to promote granulocyte removal from inflammatory sites are provided by studies demonstrating that synovial fluid from patients with active rheumatoid arthritis, and bronchoalveolar lavage fluid from rabbits with experimental pneumococcal pneumonia, induce neutrophil apoptosis (153–155). That there is potential to manipulate this process therapeutically is supported by the capability of anti-Fas monoclonal antibodies delivered to the lungs of mice with allergen-induced bronchial eosinophilia to decrease bronchial eosinophil counts and increase macrophage phagocytosis of eosinophils (156). This raises the crucial question of what effect anti-Fas monoclonal antibodies delivered to the lungs of mice has on alveolar macrophage apoptosis, alveolar epithelial cell apoptosis, pulmonary EC apoptosis, etc. If we electively utilized such a therapy, could we potentially worsen the condition?

Kawasaki and co-workers (139) reported on the beneficial effects of administration of a caspase 3 inhibitor (Z-VAD.fmk) to mice receiving intravenous LPS. Z-VAD.fmk appeared to suppress caspase 3 activity in lung tissues and prolong survival (139). However, the conclusions drawn from this important study must be treated cautiously. It is ironic that the combined effects of LPS and a caspase inhibitor (both delaying PMN apoptosis) confer greater survival advantages than the administration of LPS alone in a model of lung injury in which neutrophil-mediated tissue damage is pre-eminent. We have to reconcile theoretical and practical discrepancies such as these before we can embrace apoptotic modulation as a viable clinical tool. This reconciliation necessarily demands our attention and will provide for much intensive investigation and debate in the future.

CONCLUSION

Definitive evidence of the occurrence of widespread cellular apoptosis in patients suffering SIRS/MODS has been sparsely documented (148–150). Further data pertaining to this phenomenon is of obvious necessity and may quite conceivably be the major breakthrough required in the treatment of these disease processes. The intrinsic complexities of human SIRS/MODS make it likely that effective strategies will require a multifactorial approach combining methodologies addressing both pro- and anti-apoptotic mechanisms, ultimately dependent on specific cell and organ conditions and individual clinical circumstances. Because the most recent pertinent works (149, 150) demonstrate expansive target organ apoptotic activity in SIRS/MODS, the fundamental question may therefore be, is MODS the result of massive cellular apoptosis, or is massive cellular apoptosis the result of MODS?

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APPENDIX 1: ABBREVIATIONS COMMONLY USED IN APOPTOSIS

AIF	Apoptosis-inducing factor
Apaf-1	Apoptosis-activating factor-1
CAD	Caspase-activated deoxyribonuclease
c-IAP	Cellular inhibitors of apoptosis
CRADD	Caspase and RIP adaptor protein with a death domain
DcR1	Decoy receptor 1
DD	Death domain
DED	Death effector domain
DR	Death receptor
FADD	Fas-associated DD
FasL	Fas ligand
FLIP	FLICE-inhibitory protein
ICE	Interleukin-1β-converting enzyme
IKK	I-κB kinase α
JNK	Jun N-terminal kinase
JNKK	JNK kinase
LPS	Lipopolysaccharide
MAP kinase	Mitogen-activated protein kinase
MEKK1	MAP/Erk kinase kinase 1
NIK	NF-κB inducing kinase
PARP	poly-(ADP-ribose) polymerase
PKC	Protein kinase C
PTK	Protein tyrosine kinase
RAIDD	RIP-associated ICH-1 homologous protein with a DD
RIP	Receptor interacting protein
ROS	Reactive oxygen species
sFas	Soluble Fas
sFasL	Soluble Fas ligand
TNF	Tumor necrosis factor
TNFR	TNF receptor
TRADD	TNFR-associated protein with a DD
TRAF	TNF receptor-associated factor
TRAIL	TNF-related apoptosis-inducing ligand
TRID	TRAIL receptor without an intracellular domain
TRIP	TRAF-interacting protein